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SYSTEMATIC SIGNIFICANCE OF STIPE ANATOMY IN PENINSULAR MALAYSIAN *Blechnum* L. (BLECHNACEAE) SPECIES

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ABSTRACT

A study on the stipe anatomy was conducted on six taxa of *Blechnum* in Peninsular Malaysia namely *B. finlaysonianum, B. fraseri, B. indicum, B. melanocaulon* subsp. *pallen, B. orientale* and *B. vestitum*. The objective of this study is to investigate the variations in the stipe anatomical characteristics that can be used for species identification and classification. There are five stipe anatomical characteristics found in this study that can be used to distinguish the *Blechnum* species. The characters are stipe outlines, patterns of sclerenchyma cell layers present under epidermal cells, presence and absence of sclerenchyma cell layer encircled the vascular bundles, number of vascular bundles, and presence of auricles on the adaxial side of stipe. Similarities found in stipe anatomical characteristics in all *Blechnum* species studied are the *Aspidium* stele type and presence of sclerenchyma cell layers underneath the epidermal layer. The diagnostic anatomical characteristics found in this study is the absence of sclerenchyma cells layer ensheathing each vascular bundles in the steles of *B. finlaysonianum.* Therefore, the stipe anatomical characteristics can be used in the identification of species in the genus *Blechnum*.

Key words: Stipe anatomy, *Blechnum,* Peninsular Malaysia

ABSTRAK

Satu kajian anatomi stip telah dijalankan ke atas enam takson *Blechnum* di Semenanjung Malaysia iaitu *B. finlaysonianum, B. fraseri, B. indicum, B. melanocaulon* subsp. *pallen*, *B. orientale,* dan *B. vestitum*. Objektif kajian ini ialah untuk mengenalpasti variasi ciri anatomi stip yang boleh digunakan untuk pengecaman dan pengelasan spesies. Terdapat lima ciri anatomi stip yang boleh digunakan untuk membezakan spesies *Blechnum* yang dikaji. Ciri tersebut ialah bentuk luaran stip, corak lapisan sel sklerenkima yang hadir di bawah lapisan sel epidermis, jumlah berkas vaskular, kehadiran dan ketidakhadiran lapisan sel sklerenkima mengelilingi setiap berkas vaskular dan kehadiran struktur bak sayap/telinga pada bahagian adaksial stip. Ciri persamaan anatomi yang dijumpai dalam semua spesies *Blechnum* yang dikaji ialah kehadiran stel jenis *Aspidium* dan kehadiran lapisan sel sklerenkima di bawah lapisan sel epidermis. Ciri anatomi diagnostik yang ditemui dalam kajian ialah ketidakhadiran lapisan sel sklerenkima mengelilingi berkas vaskular pada stel dalam *B. finlaysonianum*. Oleh yang demikian, ciri anatomi stip boleh digunakan untuk pengecaman spesies dalam genus *Blechnum*.

Kata kunci: Anatomi stip, *Blechnum*, Semenanjung Malaysia

INTRODUCTION

Ferns or pteridophytes are a unique group of plants that were placed within the division Pteridophyta because they are different from flowering plants in which they dispersed using spores instead of seeds (Steve, 1974; Jones *et al*., 1984). Holttum (1968) stated that ferns can be divided into two major groups which are the true ferns and the fern allies. Plants that are included in the lycophytes (less than 1% of extant vascular plants) and monilophytes (ferns) clades are all spore-bearing or "seed-free", and due to this common features all of their members have been grouped together historically under

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various terms, such as "pteridophytes" and "ferns and fern allies" (Smith *et al*., 2006). According to Gensel and Berry (2001), lycophytes or club mosses and related plants are regarded as a distinct lineage of vascular plants with a long evolutionary history. Pryer *et al*. (2004) stated that all monilophytes are spore bearing like lycophytes. However, in the broad-scale phylogenetic relationships of vascular plants, the terms monilophytes (all ferns plus whisk ferns and horsetails) and lycophytes (including clubmosses, quillworts and spikemosses). Which specify clade membership to the terms "pteridophytes" and "ferns and fern allies" that unite these paraphyletic assemblages of plants.

Blechnaceae, the name for this family, is derived from the Greek word, 'blechnon' which generally refers to ferns (Nelson, 2000). Blechnaceae which is classified in phylum Monilophyta is a large family of nine genera and composed of an estimated 200 species (Smith *et al.,* 2006). Four of the genera can be found in Peninsular Malaysia, namely as *Blechnum, Brainea, Stenochlaena* and *Woodwardia* (Parris *et al.,* 2010). *Blechnum* is the largest genus in the family Blechnaceae (Ruiz & Garcea, 2009). According to Ruiz and Garcea (2009), species belonging to this genus can be found mainly in tropical areas. Many species are also found in temperate, warm temperate, sub-alpine and alpine areas. Some species are of tropical oceanic islands, but most species are distributed in the Southern Hemisphere (Chambers & Farrant, 2001). There are 11 *Blechnum* species in Malaysia, and in Peninsular Malaysia alone six species has been recorded (Holttum, 1968; Piggott, 1988; Chambers & Farrant, 2001). Most publications reported the chromosome number ranging from 28 to 40 (Chambers & Farrant, 2001). Polyploidization (tetraploidy and triploidy) has been reported in several taxa (Chambers & Farrant, 2001). The frond is either monomorphic or dimorphic and large, generally more than 30 cm, often exceeding 1 m in length (Cranfill, 1993). Species of this family often have dimorphic fronds where there are significant differences in fertile and sterile fronds (Large & Braggins, 2004).

The species belonging to this family are terrestrial or epilithic, and rarely found as climbing epiphytes. The stem is creeping, erect, or climbing and scaly. The adaxial surface is grooved, with vascular bundles usually arranged in U-shaped pattern (Kramer *et al.,* 1990). Petiole generally has more than two vascular bundles arranged in an arc, generally scaly at least on the abaxial side (Cranfill, 1993). Rachis has a narrow groove at the adaxial part (Kramer *et al.,* 1990). Veins of the leaves are generally free and rarely anastomose. *Blechnum orientale* is one of the common species encounter in Malaysia and grew everywhere in open areas (Chamber & Farrant, 2001). In most *Blechnum* species in Peninsular Malaysia, the fertile and sterile pinnae were similar in form and size except for *B. vestitum,* where the fertile pinnae are very narrow. The pinnae venation is free or not or once to twice forked (Holttum, 1968). Sorus is linear and parallel with pinnae midrib and protected by indusium (Holttum, 1968).

According to Metcalfe and Chalk (1979), identification of species using anatomical features are very important because it can be used to determine the botanical identity of a species included in commercial products such as food material and drug industry. The identification of *Blechnum* species depends much on the leaves which make it hard to differentiate because of the rather similar leaf morphology. Hence, the anatomical data could help to identify and to distinguish species in the genus. Therefore, the main objective of this study is to examine the stipe anatomical variations and similarities in the species belonging to the genus *Blechnum* in Peninsular Malaysia that can be used as alternative characters in species identification. This study is timely as there is no previous study on the stipe anatomy ever conducted on any species in the genus *Blechnum* especially in Peninsular Malaysia.

MATERIALS AND METHODS

Fresh specimens of six *Blechnum* taxa were collected from various localities in Peninsular Malaysia (Table 1). Stipe samples were fixed in bottles containing AA solution (70% Alcohol: 30% Acetic Acid) for 48 hours before being sectioned using *Reichert* sliding microtome. Based on Ogura (1972), the upper stipe is the nearest area to the first pinnae, the lower is the nearest area to the rhizome, and in between is the middle part. Sections of stipe were stained in Safranin and Alcian green then passed through a series of 50%, 70%, 95% and 100% Alcohol, and lastly the sections were mounted on slide using Euparal. The sections were photographed using a video camera (JVC) attached to a Leica Diaphlan microscope and images were processed using Docu Analysis Software (Sass, 1958).

RESULTS AND DISCUSSION

Stipe anatomy is suitable for plant systematic study due to the variations in the stele types and shape, and in the presence of sclerenchyma cells and many other anatomical characteristics (Ogura, 1972). Ogura (1972) also stated that stipe can have more than one stele, and can be differentiated from the amount, the shape and also the arrangement of vascular bundles. Ogura (1972) and Noraini *et al.*

Table 1. List of *Blechnum* species and samples studied

Taxa	Specimen No	Locality	Date of Collection	Collectors
B. finlaysonianum Wall. ex Hook. & Grev.	NF01	Bangi Forest Reserve, Selangor	07.02.2011	Nor Fairuz Ramli, Ruzi Abdul Rahman
B. fraseri (A. Cunn.) Luerss	NF08	Mount Berinchang, Cameron Highland, Pahang	19.02.2011	Nor Fairuz Ramli, Razali Jaman, Ruzi Abdul Rahman
B. indicum Burm. F.	NF11	Jambu Bongkok Recreational Forest, Terengganu	20.03.2011	Sani Miran
B. melanocaulon subsp. *pallen* (Brack) T.C. Chambers & P.A. Farrent	NF10	Mount Berinchang, Cameron Highland, Pahang	19.02.2011	Nor Fairuz Ramli, Razali Jaman, Ruzi Abdul Rahman
B. orientale L.	NF04	Lata Iskandar, Tapah, Perak	07.01.2011	Nor Fairuz Ramli, Ruzi Abdul Rahman
B. vestitum (Blume) Kuhn	NF06	Mount Berinchang, Cameron Highland, Pahang	19.02.2011	Nor Fairuz Ramli, Razali Jaman, Ruzi Abdul Rahman

(2012) reported that, to observe the patterns of the stele in any fern species, cross sections of stipe need to be done at the lower, middle and upper parts. In this study, variations are found in the stipe outline at the lower, middle and also at the upper part of the stipe cross sections (Figures 1-3). Therefore, this observation support the study by Ogura (1972) and Noraini *et al.* (2012) that the length of the stipe displays different patterns of the steles at the lower, middle and upper parts in some species and show similar pattern in others.

All the species studied shared a common feature of having similar types of stele. They have complex stele which consists of main vascular bundles together with additional vascular bundles. The main vascular bundle is categorized as *Onoclea* type (hippocampus shape), whereas the additional vascular bundle is categorized as *Ophioglossum* type (collateral). The overall type of stele in the species studied is *Aspidium*. Ogura (1972) stated that this type of stele consists of more than three meristeles arranged in an arch, with two larger, main vascular bundles at the adaxial part, and a hooked hippocampus xylem on the adaxial surfaces whereas, additional vascular bundles are arranged in round or elliptic pattern. *Aspidium* type was believed to be derived from the *Onoclea* type through further division (Ogura, 1972). According to Ogura (1972), this character can be found in species belonging to the genera *Aspidium, Polystichum, Dryopteris* and *Blechnum*. Therefore, this finding supported that of Ogura (1972) and proved that this character is common in all species belonging to the genus *Blechnum.* The types of stipe outline and numbers of vascular bundles present in the stipe of *Blechnum* species studied are shown in Table 4.

For convenience and to avoid lengthy descriptions, the following structures have been divided into readily recognizable groups, which are illustrated by photomicrographs or drawings that are, types of stipe outline in *Blechnum* species studied (Table 2), and patterns of sclerenchyma cells present in *Blechnum* species studied (Table 3).

In this study, six types of stipe outline were identified and the descriptions are given in Table 2. *Blechnum finlaysonianum* and *B. indicum* have Type 1 outline for the lower, middle and upper parts of stipe. *Blechnum fraseri*, on the other hand has Type 6 for the upper and middle stipes and Type 5 for the lower stipe. *Blechnum melanocaulon* subsp. *pallen* has Type 3 for lower, middle and upper stipes. *Blechnum orientale* has Type 2 for the lower, middle and upper stipes. Meanwhile, *B. vestitum* has Type 5 for the lower stipe, Type 6 for the middle and upper stipes (Table 4; Fig. 1-3). Results showed that the stipe outline can be very useful in species identification and differentiation in the genus *Blechnum*. The taxonomic significance of stipe outline in species differentiation and identification have already been shown in previous study by Noraini *et al.* (2012) in the genus *Davallia* and Maideen *et al*. (2013) in the genus *Selaginella*.

Several layers of sclerenchyma cell are present underneath adaxial and abaxial epidermises in the stipe cross sections (Figures 4A-4C). The variations of the sclerenchyma cell underneath adaxial and abaxial epidermises can be clearly seen on the upper, middle and lower stipes of the six taxa of

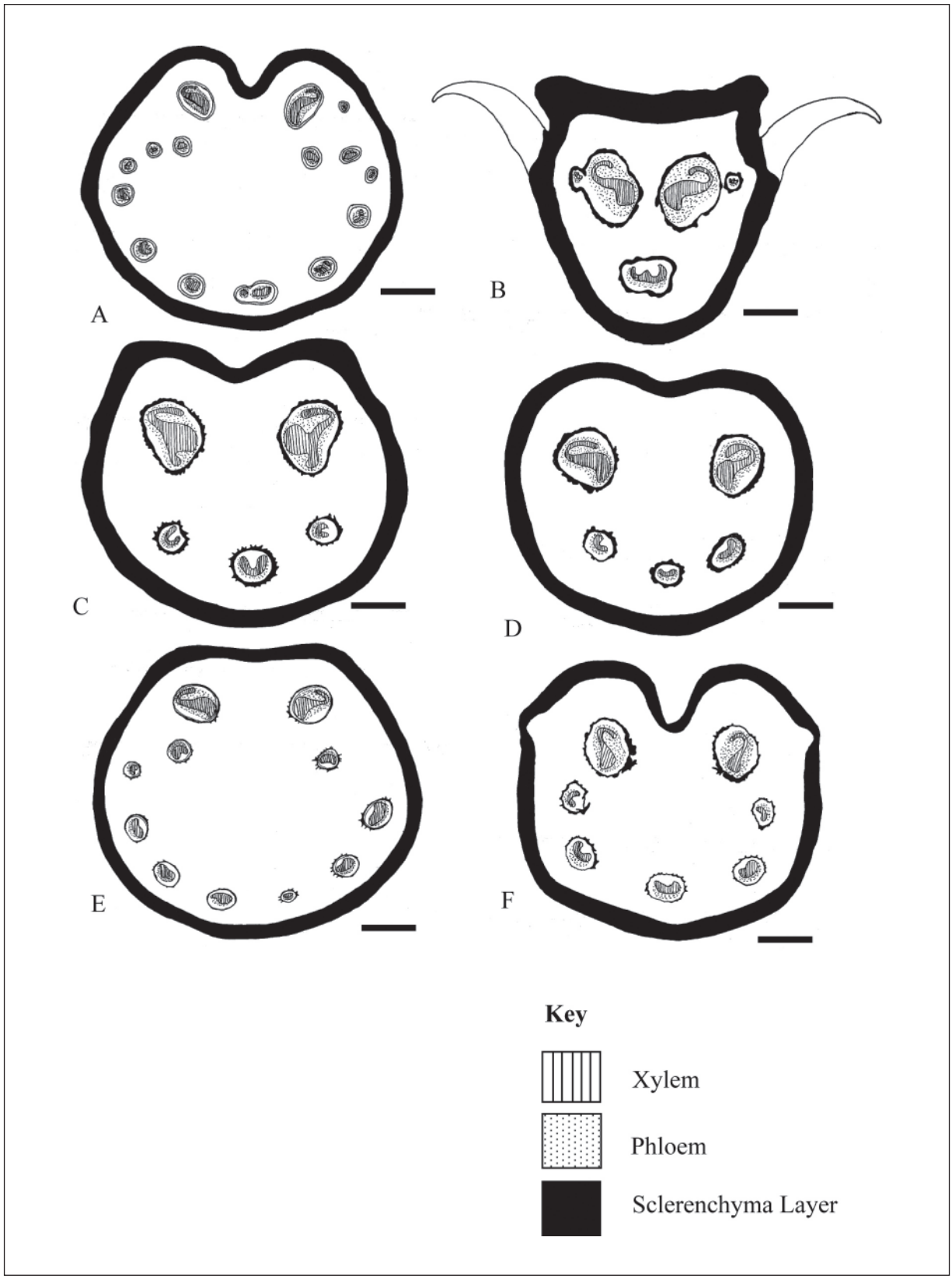


Fig. 1: Cross section of upper stipes: A) *B. finlaysonianum,* B) *B. fraseri*, C) *B. indicum*, D) *B. melanocaulon* subsp. *pallen,* E) *B. orientale,* F) *B. vestitum.*
Scale: A – F = 500 μm

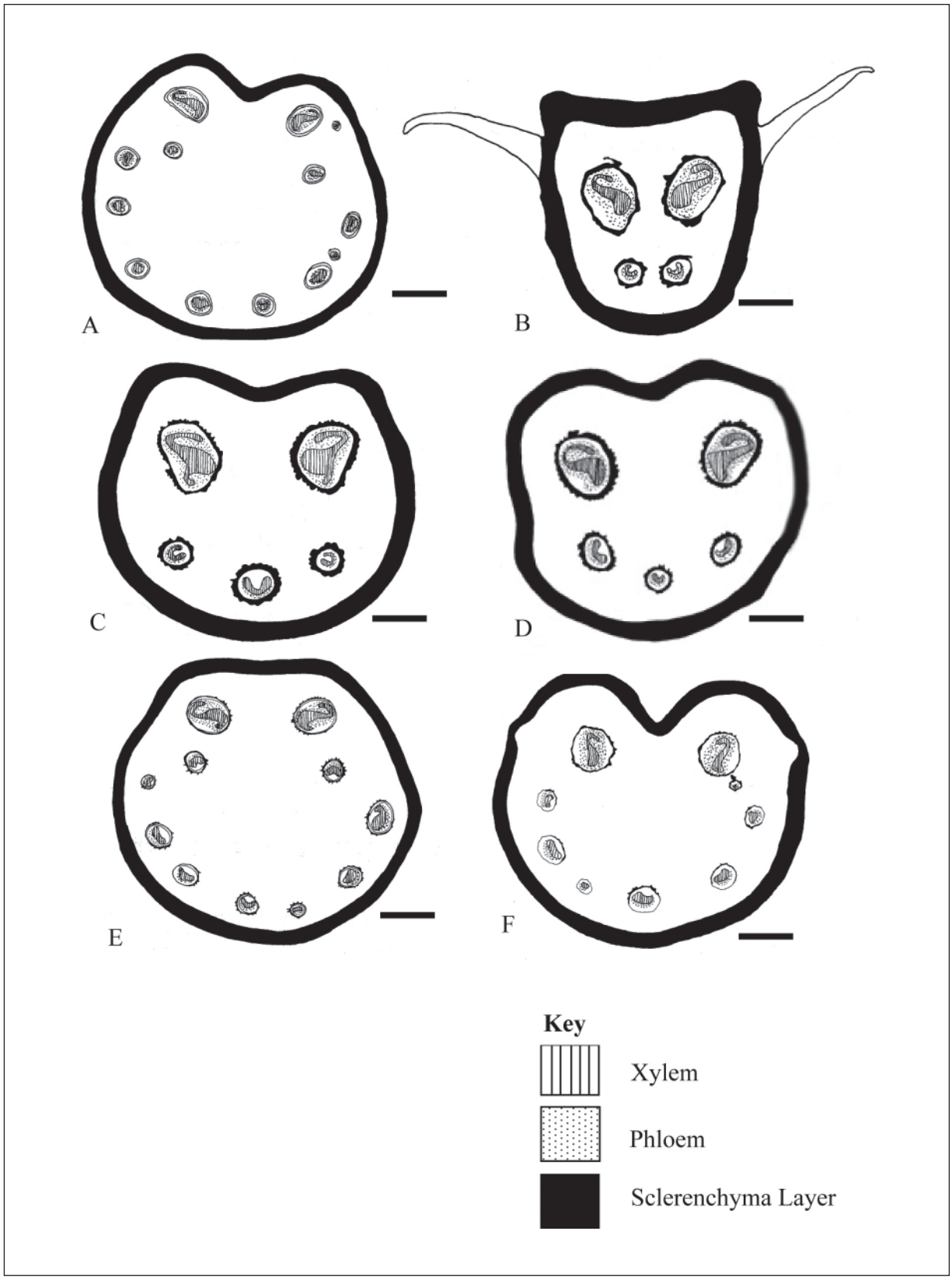


Fig. 2: Cross section of middle stipes: A) *B. finlaysonianum,* B) *B. fraseri*, C) *B. indicum*, D) *B. melanocaulon* subsp. *pallen,* E) *B. orientale,* F) *B. vestitum.*
Scale: A – F = 500 μm

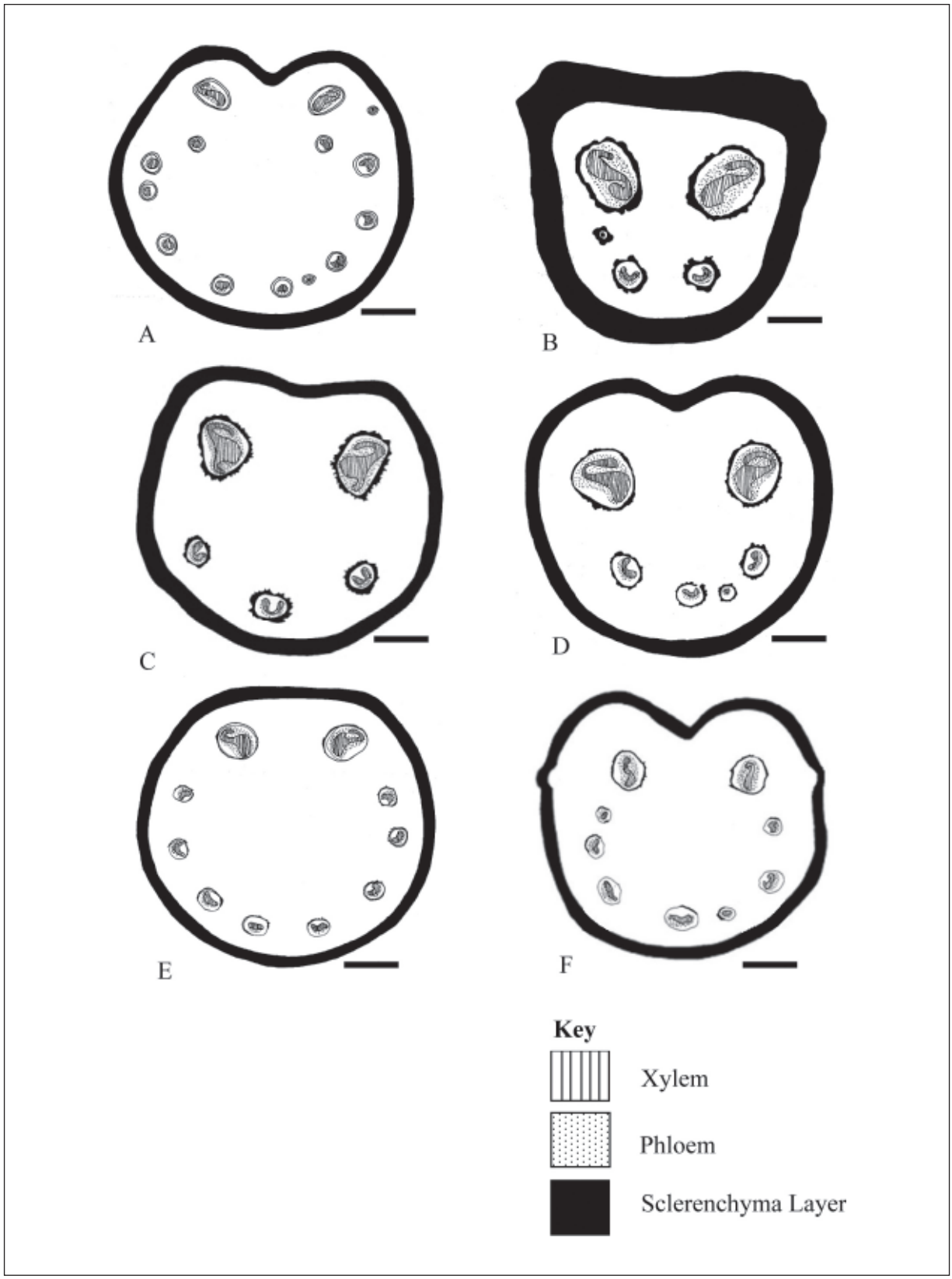


Fig. 3: Cross section of lower stipes: A) *B. finlaysonianum,* B) *B. fraseri*, C) *B. indicum*, D) *B. melanocaulon* subsp. *pallen,* E) *B. orientale,* F) *B. vestitum*.
Scale: A – F = 500 μm

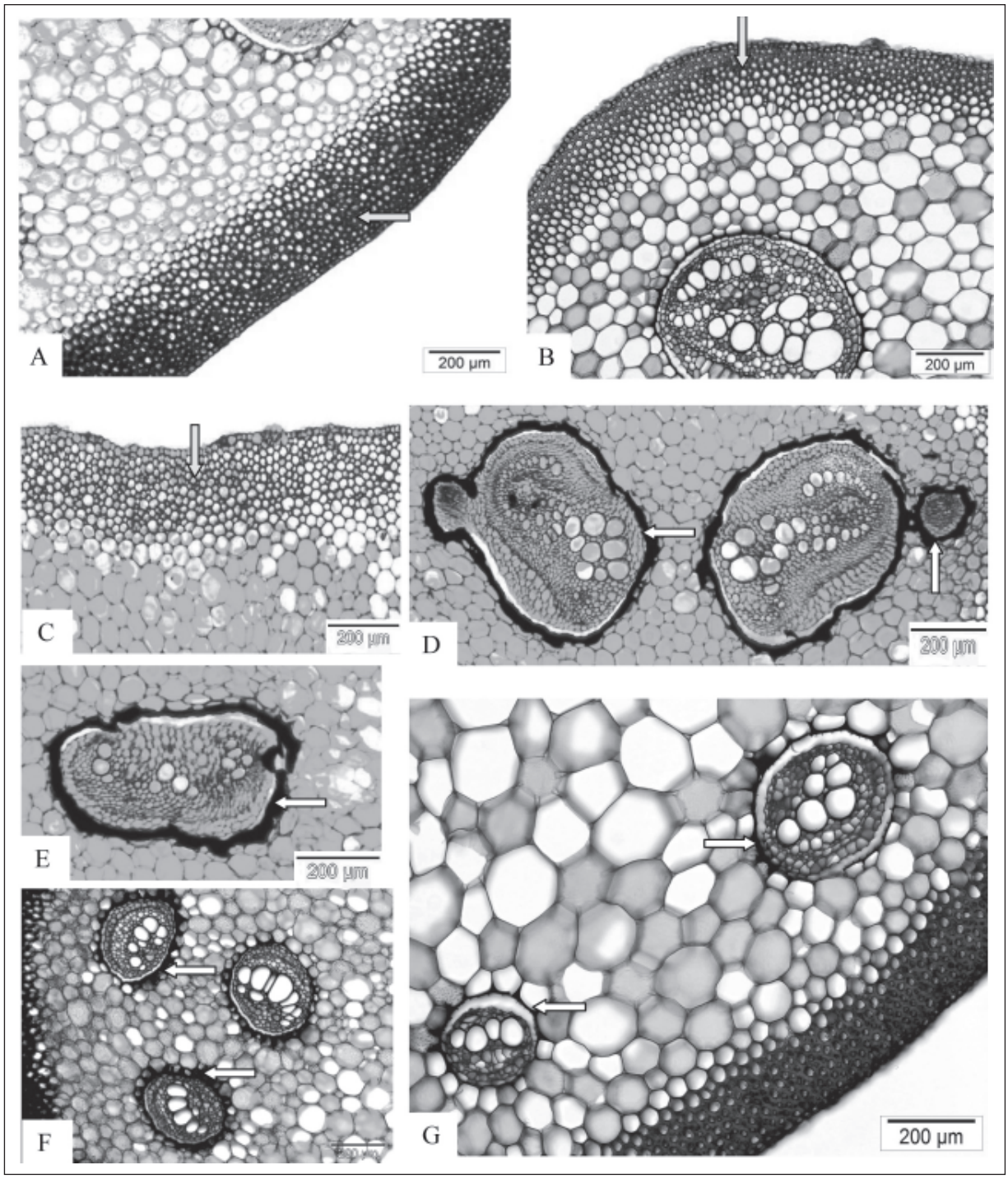


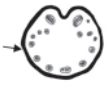
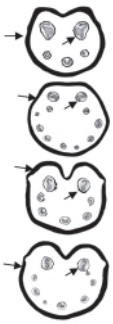
Fig. 4: Stipes cross sections: Presence of sclerenchyma cells underneath epidermal cells (yellow arrow) in A) *B. finlaysonianum*, B) *B. fraseri*, C) *B. vestitum*. Presence of sclerenchyma cells ensheathing the vascular bundles (white arrow) in D) *B. fraseri*, E) *B. indicum*, F) *B. orientale*, F) *B. vestitum*.

Blechnum studied (Fig. 1-3). Three patterns of sclerenchyma cell were observed in the stipe cross sections of *Blechnum* species studied (Table 3). Pattern 1, present in the lower, middle and upper stipes of *B. finlaysonianum*. Pattern 2, present in the lower, middle and upper stipes of *B. indicum* and *B. melanocaulon,* whereas in the upper, middle and lower stipes of *B. vestitum* and in *B. fraseri* only present in the lower stipe. Pattern 3 is present in the upper and middle stipes of *B. fraseri.* These results have shown that some species such as *B. finlaysonianum, B. fraseri, B. orientale* and *B. vestitum* can be differentiated from the other *Blechnum* species found in Peninsular Malaysia by the patterns of sclerenchyma cell presence underneath epidermal cells and surrounding vascular

Table 2. Types of stipe outline in *Blechnum* species studied

Type of stipe	Description	Illustrations
1	Adaxial: convex V-shaped (hump present) Abaxial: ¾ of circle	
2	Adaxial: almost flat to flat (hump absent) Abaxial: ¾ of circle	
3	Adaxial: convex wide with shallow U-shaped (hump present) Abaxial: U-shaped	
4	Adaxial: convex V-shaped (hump present) Abaxial: U-shaped	
5	Adaxial: curve Abaxial: U-shaped With a flat sides surface on adaxial and abaxial sides	
6	Adaxial: curve Abaxial: wide U-shaped With a rounded abaxial side and projection forming lobes, lobe length 700-1500 µm and 300-350 µm wide.	

Table 3. Patterns of sclerenchyma cells present in *Blechnum* species studied

Illustrations	Pattern Type	Description
	1	Several layers of sclerenchyma cells present continuously under epidermal cells (arrow), no layer of sclerenchyma cells ensheathing the vascular bundles.
	2	Several layers of sclerenchyma cells present continuously under epidermal cells (arrow), at the auricles, layer of sclerenchyma cells ensheathing the vascular bundles (arrow).
	3	Several layers of sclerenchyma cells present continuously under epidermal cells (arrow) but absent at the auricles, layer of sclerenchyma cells ensheathing the vascular bundles (arrow).

bundles. The other two taxa, *B. indicum* and *B. melanocaulon* subsp. *pallen* resemble one another by having similar pattern of sclerenchyma cell at the lower, middle and upper stipes. The patterns of sclerenchyma cell in the stipe of all species studied are given in Table 5.

The presence of complete and incomplete black layers surrounding the steles have been observed in almost all species studied. According to Noraini *et al.* (2012) and Ogura (1972), the layer is actually layers of sclerenchyma cells. In this study, these layers are present in all *Blechnum* species studied except in *B. finlaysonianum.* The sclerenchyma cell layers are common in many fern species such as shown in *Pteridium aquilinum* and *Pyrossia lingua* (Ogura 1972), in many *Davallia* species (Noraini

Table 4. Types of stipe outline and number of vascular bundles present in the stipe of *Blechnum* species studied

Taxa	Upper stipe		Middle stipe		Lower stipe	
	Type of stipe outline	Number of vascular bundles	Type of stipe outline	Number of vascular bundles	Type of stipe outline	Number of vascular bundles
B. finlaysonianum	1	15	1	13	1	14
B. fraseri	6	3	6	4	5	5
B. indicum	1	5	1	5	1	5
B. melanocaulon subsp. *pallen*	4	5	6	5	4	6
B. orientale	2	11	3	11	3	10
B. vestitum	6	7	5	9	6	9

Table 5. Patterns of sclerenchyma cells and presence of auricles in *Blechnum* species studied

Taxa	Upper stipe		Middle stipe		Lower stipe	
	Auricles	Pattern of sclerenchyma cells	Auricles	Pattern of sclerenchyma cells	Auricles	Pattern of sclerenchyma cells
B. finlaysonianum	Absent	1	Absent	1	Absent	1
B. fraseri	2	3	2	3	Absent	2
B. indicum	Absent	2	Absent	2	Absent	2
B. melanocaulon subsp. *pallen*	Absent	2	2	2	Absent	2
B. orientale	Absent	2	Absent	2	Absent	2
B. vestitum	2	2	2	2	2	2

et al., 2012) and *Selaginella* species (Maideen *et al.,* 2013). Thus, the presence and absence of sclerenchyma cell layers surrounding the steles in stipe can be used for species differentiation within the genus *Blechnum*. The sclerenchyma cell layers present ensheathing the vascular bundles in *B. fraseri, B. indicum, B. orientale* and *B. vestitum* are shown in Fig. 4D-G.

The presence and absence of auricles at the adaxial side of stipe could be used to differentiate certain taxa such as *B. melanocaulon* subsp. *pallen* where the auricles only present at the middle stipes, but in *B. vesticum* the auricles present at the upper, middle and lower stipes. The three species (*B. finlaysonianum, B. indicum, B.orientale*) have no auricles (Table 5), whilst in *B. fraseri* the auricles present at the upper and middle stipes.

CONCLUSIONS

Six forms of stipe outline are found on *Blechnum finlaysonianum* and *B. indicum* have similar forms of stipe in the middle, upper and lower parts whereas *B. fraseri*, *B. melanocaulon* subsp. *pallen*, *B. orientale* and *B. vestitum* have two forms of stipe. Three patterns in the presence of sclerenchyma cells in the stipe cross sections have been recorded in this study. The presence of auricles can be used to identify *B. melanocaulon* subsp. *pallen* with certainty. The stipe anatomical characteristics have proven to be useful in species differentiation and identification in the genus *Blechnum*. The anatomical characters observed in this study revealed a number of interesting features with some characteristics could be of taxonomic and diagnostic values particularly at species level. The anatomical data obtained from the present study are used in the construction of an artificial identification key to the species of *Blechnum* as given below.

Key to the *Blechnum* in Peninsular Malaysia

1. Sclerenchyma cells pattern in the upper stipe of type 1 2
1. Sclerenchyma cells pattern in the upper stipe of type 2 3
2. Auricles absent at the upper, middle and lower stipes; sclerenchyma cells pattern at the lower stipe of type 1 .....*B. finlaysonianum*
2. Auricles present at the upper and middle stipes; sclerenchyma cells pattern at the lower stipe of type 2 .....*B. fraseri*

3. Outline of upper stipe is type 1 or 4 with five vascular bundles at upper stipe 4
3. Outline of upper stipe is type 2 or 6 with more than five vascular bundles at the upper stipe 5
4. Outline of middle stipe type 1 and outline of lower stipe type 1 .....*B. indicum*
4. Outline of middle stipe type 6 and outline of lower stipe type 4 .....*B. melanocaulon* subsp. *pallen*
5. Auricles absent on both sides of stipe from upper to lower part; type of stipe at upper stipe is type 2; with 11 vascular bundles .....*B. orientale*
5. Auricles present at both side from upper to lower part, type of stipe at upper stipe is type 6; with seven vascular bundles .....*B. vestitum*

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